

**Sample preparation:** Filter (Ultrafree MC) plasma at 6000 g for 10 min, inject a 10  $\mu$ L aliquot of the ultrafiltrate.

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**HPLC VARIABLES**

**Column:** 150  $\times$  3.9 4  $\mu$ m Nova Pak C18

**Mobile phase:** 200 mM pH 7.2 borate buffer (Prepare the buffer by dissolving 12.4 g boric acid in 1 L water and adjusting pH to 7.2 with 1 M NaOH.)

**Flow rate:** 1

**Injection volume:** 10

**Detector:** UV 300

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**CHROMATOGRAM**

**Retention time:** 4.44

**Limit of detection:** 30 ng/mL

**Limit of quantitation:** 80 ng/mL

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**KEY WORDS**

pharmacokinetics; plasma

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**REFERENCE**

Garcia-Capdevila,L.; López-Calull,C.; Arroyo,C.; Moral,M.A.; Mangues,M.A.; Bonal,J. Determination of imipenem in plasma by high-performance liquid chromatography for pharmaceutical studies in patients, *J.Chromatogr.B*, **1997**, 692, 127–132.

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**SAMPLE**

**Matrix:** solutions

**Sample preparation:** Inject a 10  $\mu$ L aliquot of a 400  $\mu$ g/mL solution.

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**HPLC VARIABLES**

**Column:** 150  $\times$  4.6 Microsorb C8 80-315

**Mobile phase:** 1 mM  $\text{KH}_2\text{PO}_4$  adjusted to pH 6.8 with 500 mM NaOH

**Flow rate:** 1.5

**Injection volume:** 10

**Detector:** UV 300

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**REFERENCE**

Connolly,M.; Debenedetti,P.G.; Tung,H.-H. Freeze crystallization of imipenem, *J.Pharm.Sci.*, **1996**, 85, 174–177.

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# Imipramine

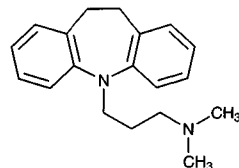
**Molecular formula:**  $\text{C}_{19}\text{H}_{24}\text{N}_2$

**Molecular weight:** 280.41

**CAS Registry No.:** 50-49-7, 113-52-0 (HCl)

**Merck Index:** 4955

**Lednicer No.:** 1 401; 2 420; 3 32; 4 146 201 203



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**SAMPLE**

**Matrix:** blood

**Sample preparation:** Add 250  $\mu$ L 2 M sodium carbonate to 500  $\mu$ L plasma. Add 100  $\mu$ L 1  $\mu$ g/mL IS in MeOH, extract with 10 mL n-hexane. Shake for 30 min and centrifuge at 3000 g for 10 min. Cool in a dry ice-acetone bath. Add 200  $\mu$ L 0.3% phosphoric acid to the upper organic layer. Shake for 10 min and centrifuge at 3000 g for 10 min. Separate the organic layer. Inject a 100  $\mu$ L aliquot of the acidic aqueous layer.

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**HPLC VARIABLES**

**Column:** 250  $\times$  4.6 5  $\mu$ m C18 Symmetry (Waters Millipore, USA)

**Mobile phase:** MeCN:67 mM potassium phosphate buffer adjusted to pH 3.0 with phosphoric acid 35:65 (After each chromatographic session wash the column with 200 mL MeCN:water 50:50.)

**Flow rate:** 1.2

**Injection volume:** 100

**Detector:** UV 226, UV 254, UV 400

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#### CHROMATOGRAM

**Retention time:** 9.84

**Internal standard:** clovoxamine (6.5)

**Limit of quantitation:** 5 ng/mL (UV 226, UV 400); 7 ng/mL (UV 254)

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#### OTHER SUBSTANCES

**Extracted:** metabolites, amitriptyline, clomipramine, desipramine, fluoxetine, maprotiline, nortriptyline

**Simultaneous:** amineptine, chlordiazepoxide, chlorpromazine, clonazepam, clorazepate, clozapine, cyamemazine, desmethylmaprotiline, desmethylvenlafaxine, doxepin, flunitrazepam, haloperidol, levomepromazine, lorazepam, loxapine, mianserine, sulpiride, trimipramine, venlafaxine, viloxazine, zolpidem, zopiclone

**Noninterfering:** diazepam, valproic acid

**Interfering:** carbamazepine, fluvoxamine

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#### KEY WORDS

plasma

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#### REFERENCE

Aymard, G.; Livi, P.; Pham, Y.T.; Diquet, B. Sensitive and rapid method for the simultaneous quantification of five antidepressants with their respective metabolites in plasma using high-performance liquid chromatography with diode-array detection, *J. Chromatogr. B*, **1997**, *700*, 183–189.

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#### SAMPLE

**Matrix:** blood

**Sample preparation:** 2 mL Plasma + 1600 ng clomipramine in MeOH + 2 mL 1 M NaOH + 5 mL hexane:isoamyl alcohol 99:1, shake mechanically for 15 min, centrifuge at 1686 g for 5 min. Remove the organic phase and add it to 200  $\mu$ L 0.05% orthophosphoric acid, shake for 15 min, centrifuge for 5 min, inject a 50  $\mu$ L aliquot of the aqueous phase.

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#### HPLC VARIABLES

**Guard column:**  $\mu$ Bondapak/Porasil

**Column:**  $\mu$ Bondapak C18

**Mobile phase:** MeCN:buffer 40:60 (Buffer was 13.68 g  $\text{KH}_2\text{PO}_4$  in 2 L water, adjusted to pH 4.7 with dilute KOH.)

**Column temperature:** 50

**Flow rate:** 2

**Injection volume:** 50

**Detector:** UV 254

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#### CHROMATOGRAM

**Retention time:** 5

**Internal standard:** clomipramine (7.5)

**Limit of detection:** 0.6 ng

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#### OTHER SUBSTANCES

**Extracted:** amitriptyline, desipramine, nortriptyline

**Simultaneous:** chlordiazepoxide, chlorpromazine, cimetidine, clomipramine, diazepam, doxepin, flurazepam, lorazepam, oxazepam, pentobarbital, perphenazine, phenobarbital, phenytoin, prochlorperazine, propoxyphene, secobarbital, thioridazine, trifluoperazine

**Noninterfering:** acetaminophen, codeine, meperidine

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#### KEY WORDS

plasma

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**REFERENCE**

Wong, S.H.Y.; McCauley, T. Reversed phase high-performance liquid chromatographic analysis of tricyclic anti-depressants in plasma, *J.Liq.Chromatogr.*, **1981**, *4*, 849–862.

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**SAMPLE**

**Matrix:** blood

**Sample preparation:** 10 mL Plasma or whole blood + 1 mL 1 M NaOH, extract twice with 10 mL hexane for 30 min. Remove the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 1 mL 100 mM HCl, add 5 mL chloroform, vortex for 1 min, centrifuge. Remove a 4.5 mL aliquot of the organic layer and evaporate it to dryness, reconstitute the residue in 100  $\mu$ L mobile phase, inject a 50  $\mu$ L aliquot. (It is implied, but not explicitly stated in the paper, that this extraction procedure works for this compound.)

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**HPLC VARIABLES**

**Column:** 10  $\mu$ m Micropak CN (Varian)

**Mobile phase:** MeCN:20 mM ammonium acetate 90:10

**Flow rate:** 2.5

**Injection volume:** 50

**Detector:** UV 254

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**CHROMATOGRAM**

**Retention time:** 10.1

**Limit of detection:** 10 ng/mL

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**OTHER SUBSTANCES**

**Simultaneous:** acetophenazine, amitriptyline, benztropine, butaperazine, carphenazine, chlorpromazine, fluphenazine, haloperidol, mesoridazine, nortriptyline, orphenadrine, piperacetazine, promethazine, thioridazine, trifluoperazine, triflupromazine, trihexyphenidyl, trimeprazine

**Interfering:** promazine, thiothixene

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**KEY WORDS**

plasma; whole blood

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**REFERENCE**

Curry, S.H.; Brown, E.A.; Hu, O.Y.-P.; Perrin, J.H. Liquid chromatographic assay of phenothiazine, thioxanthene and butyrophenone neuroleptics and antihistamines in blood and plasma with conventional and radial compression columns and UV and electrochemical detection, *J.Chromatogr.*, **1982**, *231*, 361–376.

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**SAMPLE**

**Matrix:** blood

**Sample preparation:** 1 mL Serum + 200  $\mu$ L 10  $\mu$ g/mL protriptyline in water + 200  $\mu$ L 80 g/L NaHCO<sub>3</sub> + 5 mL hexane, vortex for 15 s, centrifuge for 5 min. Remove the hexane layer and evaporate it in a stream of nitrogen at 60°. Reconstitute in 100  $\mu$ L mobile phase, vortex for 15 s, inject a 50  $\mu$ L aliquot.

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**HPLC VARIABLES**

**Column:** 300  $\times$  4 10  $\mu$ m  $\mu$ Bondapak CN

**Mobile phase:** MeCN:MeOH:5 mM phosphate buffer 60:15:25, adjusted to pH 7.0

**Flow rate:** 2

**Injection volume:** 50

**Detector:** UV 254

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**CHROMATOGRAM**

**Retention time:** 3.62

**Internal standard:** protriptyline (12.20)

**Limit of detection:** 6 ng/mL

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**OTHER SUBSTANCES**

**Simultaneous:** trimipramine, doxepin, amitriptyline, desmethyldoxepin, nortriptyline, desipramine, chlorpromazine, procainamide, thioridazine, propranolol, propoxyphene, disopyramide, maprotiline

**Noninterfering:** caffeine, theophylline, salicylic acid, chlordiazepoxide, methaqualone, diazepam, acetaminophen, trifluoperazine

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**KEY WORDS**

serum

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**REFERENCE**

Koteel,P.; Mullins,R.E.; Gadsden,R.H. Sample preparation and liquid-chromatographic analysis for tricyclic antidepressants in serum, *Clin.Chem.*, **1982**, *28*, 462-466.

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**SAMPLE**

**Matrix:** blood

**Sample preparation:** Condition a Bond-Elut C18 column with 2 volumes MeOH then 2 volumes water. Add 1 mL serum then 200  $\mu$ L 700 ng/mL promazine in MeOH:0.1 M HCl 13:87 to each column, wash with 2 volumes water, wash with 2 volumes 0.1 M acetic acid, wash with MeOH/water, add 200  $\mu$ L 10 mM ammonium acetate in MeOH, wait for 30 s, elute with vacuum, repeat elution process two more times. Combine eluates and evaporate them to dryness at 56-8° under compressed air. Reconstitute with 200  $\mu$ L mobile phase, vortex 10 s, inject 75-100  $\mu$ L aliquot. (MeOH/water was 500 mL MeOH:water 65:35 plus 25  $\mu$ L concentrated HCl.)

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**HPLC VARIABLES**

**Column:** 250  $\times$  4.6 5  $\mu$ m Supelco silica

**Mobile phase:** EtOH:MeCN:t-butylamine 98:2:0.05 (Mix 1 gallon EtOH with 77 mL MeCN and 1.9 mL t-butylamine.)

**Flow rate:** 2

**Injection volume:** 75-100

**Detector:** UV 254

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**CHROMATOGRAM**

**Retention time:** 4.0

**Internal standard:** promazine (5.2)

**Limit of detection:** 2 ng/mL

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**OTHER SUBSTANCES**

**Extracted:** amitriptyline, desipramine, desmethyldoxepin, doxepin, nortriptyline, protriptyline

**Simultaneous:** procainamide, zimeldine, morphine, codeine, trifluoperazine, desmethyldisopyramide, 10-hydroxynortriptyline, prochlorperazine, oxaprotiline, 2-hydroxydesipramine, chlorpheniramine, maprotiline, norzimeldine, iminostilbene, desmethylchlordiazepoxide, buprion, diazepam, demoxepam, chlordiazepoxide, propoxyphene, dextropropoxyphene, cocaine, oxapam, trimipramine, mianserin, trimeprazine, loxepin, fluphenazine, methadone, trifluopromazine, phenteramine, chlorimipramine, perphenazine, quinidine, thioridazine, hydroxyamoxapine, meperidine, chlorpromazine, disopyramide, amphetamine, 2-hydroxyimipramine

**Noninterfering:** thiopropazine

**Interfering:** iprindole, pyrilamine, promethazine, prolixin, amoxapine, N-acetylprocainamide

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**KEY WORDS**

serum; normal phase; SPE

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**REFERENCE**

Beierle,F.A.; Hubbard,R.W. Liquid chromatographic separation of antidepressant drugs: I. Tricyclics, *Ther.Drug Monit.*, **1983**, *5*, 279-292.

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**SAMPLE**

**Matrix:** blood

**Sample preparation:** 2 mL Plasma + 100  $\mu$ L 1  $\mu$ g/mL loxapine in isopropanol:diethylamine 99.9:0.1 + 250  $\mu$ L 25% potassium carbonate containing 0.1% diethylamine + 5 mL hexane: isoamyl alcohol 97:3, vortex for 30 s, centrifuge at 500 g for 3 min. Remove the organic layer and add it to 100  $\mu$ L 250 mM HCl, vortex for 30 s, inject a 50  $\mu$ L aliquot of the aqueous phase.

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**HPLC VARIABLES**

**Guard column:** 50  $\times$  4.6 40  $\mu$ m C8 (Supelco)

**Column:** 250 × 4.6 5 µm Supelcosil C8

**Mobile phase:** MeCN:water:diethylamine:85% phosphoric acid 53.3:45.1:1:0.4, pH adjusted to 7.2 with NaOH or phosphoric acid

**Flow rate:** 2

**Injection volume:** 50

**Detector:** UV 254

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#### CHROMATOGRAM

**Retention time:** k' 5.00

**Internal standard:** loxapine (k' 7.18)

**Limit of detection:** 2.5 ng/mL

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#### OTHER SUBSTANCES

**Extracted:** amitriptyline, chlordiazepoxide, chlorpromazine, desipramine, desmethyldiazepam, desmethylchlordiazepoxide, desmethyldoxepin, doxepin, fluphenazine, haloperidol, nortriptyline, oxazepam, thiothixene

**Noninterfering:** molindone, perphenazine, trifluoperazine

**Interfering:** diazepam

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#### KEY WORDS

plasma

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#### REFERENCE

Kiel, J.S.; Abramson, R.K.; Morgan, S.L.; Voris, J.C. A rapid high performance liquid chromatographic method for the simultaneous measurement of six tricyclic antidepressants, *J. Liq. Chromatogr.*, **1983**, 6, 2761–2773.

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#### SAMPLE

**Matrix:** blood

**Sample preparation:** 2 mL Serum or plasma + 100 µL 1 µg/mL IS in water + 0.5 mL water, vortex, extract with 10 mL toluene:isoamyl alcohol 99:1 for 10 min on a rotator, centrifuge for 5 min. Remove upper organic layer, evaporate under a stream of nitrogen at 37°, take up in 150 µL mobile phase, vortex for 2 min, add 0.5 mL hexane, vortex briefly, centrifuge for 5 min, discard upper hexane layer, inject a 100 µL aliquot of the lower layer.

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#### HPLC VARIABLES

**Column:** 250 × 4 Bio-Sil ODS-10 (Bio-Rad)

**Mobile phase:** MeCN:pH 4.5 50 mM phosphate buffer 30:70 (Buffer was 6.9 g KH<sub>2</sub>PO<sub>4</sub> in 1 L adjusted to pH 4.5 with orthophosphoric acid.)

**Column temperature:** 45

**Flow rate:** 2.5

**Injection volume:** 100

**Detector:** UV 202

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#### CHROMATOGRAM

**Retention time:** 8.4

**Internal standard:** U-31485 (6.9)

**Limit of detection:** 1 ng/mL

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#### OTHER SUBSTANCES

**Extracted:** desipramine, protriptyline

**Noninterfering:** N-acetylprocainamide, amitriptyline, caffeine, chlordiazepoxide, chlorpromazine, diazepam, flurazepam, lorazepam, oxazepam, prazepam, procainamide, propranolol, thioridazine

**Interfering:** alprazolam, nortriptyline, triazolam

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#### KEY WORDS

plasma; serum

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#### REFERENCE

McCormick, S.R.; Nielsen, J.; Jatlow, P. Quantification of alprazolam in serum or plasma by liquid chromatography, *Clin. Chem.*, **1984**, 30, 1652–1655.

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**SAMPLE****Matrix:** blood**Sample preparation:** 500  $\mu$ L Plasma + 37  $\mu$ L 2  $\mu$ g/mL IS in MeOH + 500  $\mu$ L pH 10 borate buffer + 1.5 mL hexane:isoamyl alcohol 95:5, shake for 10 min. Evaporate the organic layer to dryness under a stream of nitrogen, reconstitute in 100  $\mu$ L MeOH, inject a 50  $\mu$ L aliquot. (The borate buffer was prepared as follows. Prepare a solution of 61.8 g boric acid and 74.6 g KCl in 1 L water. Add 630 mL of this solution to 370 mL 106 g/L sodium carbonate solution. Adjust pH to 10.0 with 6 M NaOH and store at 35-37°.)

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**HPLC VARIABLES****Column:** 250  $\times$  4.6 Zorbax Sil**Mobile phase:** MeOH:ammonium hydroxide 998:2**Flow rate:** 1.5**Injection volume:** 50**Detector:** UV 254

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**CHROMATOGRAM****Retention time:** 5**Internal standard:** N-desmethyldesipramine hydrochloride (10)**Limit of quantitation:** 20 ng/mL

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**OTHER SUBSTANCES****Extracted:** amitriptyline, nortriptyline, desipramine, 2-hydroxyimipramine, 2-hydroxydesipramine, metabolites**Also analyzed:** doxepin, desmethyldoxepin, desmethyldesipramine, desipramine, maprotiline, protriptyline**Noninterfering:** chlorthalidone, diazepam, flurazepam, oxazepam, thioridazine

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**KEY WORDS**

plasma

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**REFERENCE**

Sutfin, T.A.; D'Ambrosio, R.; Jusko, W.J. Liquid-chromatographic determination of eight tri- and tetracyclic antidepressants and their major active metabolites, *Clin. Chem.*, **1984**, 30, 471-474.

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**SAMPLE****Matrix:** blood**Sample preparation:** Evaporate 200  $\mu$ L 1  $\mu$ g/mL desipramine in MeOH into a tube, add 2 mL plasma, add 2 mL pH 10 Titrisol buffer (Merck), add 8 mL diethyl ether, shake for 15 min, centrifuge at 2800 g for 5 min. Remove the organic phase and shake it with 100  $\mu$ L 50 mM phosphoric acid for 15 min, centrifuge at 2800 g for 10 s. Remove the aqueous layer and vortex it with 2 mL diethyl ether for 10 s, centrifuge at 2800 g. Discard the organic layer and inject a 10-50  $\mu$ L aliquot of the aqueous layer.

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**HPLC VARIABLES****Column:** 300  $\times$  3.9 10  $\mu$ m  $\mu$ Bondapak C18**Mobile phase:** MeCN:25 mM  $\text{KH}_2\text{PO}_4$ :water 45:50:5**Flow rate:** 1**Injection volume:** 10-50**Detector:** UV 254

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**CHROMATOGRAM****Retention time:** 8.9**Internal standard:** desipramine (13)**Limit of detection:** 2 ng/mL

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**OTHER SUBSTANCES****Simultaneous:** desipramine, trimipramine**Noninterfering:** alimemazine, alprazolam, amineptine, amitriptyline, caffeine, carbamazepine, citalopram, clobazam, desmethylflunitrazepam, diazepam, dibenzepine, estazolam, ethyl lofla-

zepate, indalpine, loprazolam, lorazepam, meprobamate, nitrazepam, norclobazam, nordiazepam, nortriptyline, oxazepam, triazolam, viloxazine

**Interfering:** monodesmethyltrimipramine, flunitrazepam, levomepromazine

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## KEY WORDS

plasma

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## REFERENCE

Pok Phak,R.; Conquy,T.; Gouezo,F.; Viala,A.; Grimaldi,F. Determination of metapramine, imipramine, trimipramine and their major metabolites in plasma by reversed-phase column liquid chromatography, *J.Chromatogr.*, **1986**, 375, 339–347.

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## SAMPLE

**Matrix:** blood

**Sample preparation:** Condition a 1 mL Analytichem cyanopropyl SPE cartridge with 1 mL water and 1 mL MeOH, do not allow to dry. Add 1 mL serum + 250  $\mu$ L 50 mM sodium n-heptanesulfonic acid to the SPE cartridge, wash with 1 mL water, 1 mL MeOH:water 50:50, air dry cartridge, elute with 1 mL MeOH:triethylamine 99.2:0.8, evaporate eluate to dryness under a stream of nitrogen at 40°, reconstitute residue with 250  $\mu$ L mobile phase, inject a 50  $\mu$ L aliquot.

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## HPLC VARIABLES

**Column:** 150  $\times$  4.6 5 $\mu$ m Spherisorb cyanopropyl

**Mobile phase:** MeOH:20 mM phosphoric acid containing 0.05% N,N-diethyloctylamine 55:45, pH was 2.4

**Flow rate:** 1.5

**Injection volume:** 50

**Detector:** UV 214

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## CHROMATOGRAM

**Retention time:** 9

**Internal standard:** imipramine

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## OTHER SUBSTANCES

**Simultaneous:** doxepin

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## KEY WORDS

serum; imipramine is IS; SPE

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## REFERENCE

Emm,T.; Lesko,L.J.; Perkal,M.B. Simultaneous determination of doxepin and nordoxepin in serum using high-performance liquid chromatography, *J.Chromatogr.*, **1987**, 419, 445–451.

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## SAMPLE

**Matrix:** blood

**Sample preparation:** Condition a Bond Elut C-18 SPE cartridge twice with MeOH and twice with water. 500  $\mu$ L Serum + 50  $\mu$ L 1  $\mu$ g/mL N-propionylprocainamide in 2.5 mM HCl, add to SPE cartridge, wash with 2 volumes water, wash with 2 volumes 0.1 M acetic acid, wash with 1 volume MeOH:2.5 mM HCl 10:90. Add 200  $\mu$ L 10 mM acetic acid and 5 mM diethylamine in MeOH to column, let stand 1 min, elute under vacuum, repeat, evaporate eluents to dryness under nitrogen at room temperature, reconstitute in 100  $\mu$ L mobile phase, inject a 40  $\mu$ L aliquot.

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## HPLC VARIABLES

**Guard column:** Pelliguard LC-CN (Supelco)

**Column:** 150  $\times$  4.6 5  $\mu$ m Supelcosil LC-PCN

**Mobile phase:** MeCN:MeOH:10 mM pH 7.0 phosphate buffer 58:14:28

**Flow rate:** 1.2

**Injection volume:** 40

**Detector:** UV 254

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**CHROMATOGRAM****Retention time:** 10.5**Internal standard:** N-propionylprocainamide (6)**Limit of quantitation:** 25 ng/mL

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**OTHER SUBSTANCES****Extracted:** amitriptyline, desipramine, doxepin, nortriptyline, protriptyline, trimipramine**Simultaneous:** atropine, butalbital, chlorpromazine, maprotiline, methadone, norpropoxyphene, phenylpropanolamine, procainamide, prochlorperazine, promethazine, propranolol, quinidine, trifluoperazine, trimeprazine**Noninterfering:** acetaminophen, allopurinol, amikacin, amoxapine, amytal, bretylium, caffeine, carbamazepine, carisoprodol, chloramphenicol, chlordiazepoxide, chlorpropamide, clonazepam, codeine, diazepam, disopyramide, droperidol, ethinamate, ethinamate, ethosuximide, fluphenazine, flurazepam, furosemide, gentamicin, haloperidol, hydrochlorothiazide, hydroxyzine, ibuprofen, kanamycin, lidocaine, loxapine, meperidine, mephobarbital, meprobamate, methaqualone, methotrexate, morphine, nafcillin, naloxone, neomycin, perphenazine, phenacetin, phenobarbital, phenytoin, prazepam, primidone, procaine, propoxyphene, reserpine, salicylamide, salicylic acid, secobarbital, spironolactone, theophylline, thiopental, thioridazine, tobramycin, valproic acid, verapamil**Interfering:** hydroxynortriptyline

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**KEY WORDS**serum; SPE

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**REFERENCE**Lin, W.-N.; Frade, P.D. Simultaneous quantitation of eight tricyclic antidepressants in serum by high-performance liquid chromatography, *Ther. Drug Monit.*, **1987**, *9*, 448–455.

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**SAMPLE****Matrix:** blood**Sample preparation:** 500  $\mu$ L Serum + 250  $\mu$ L di-iso-propyl ether:n-butyl alcohol 7:3 containing 800 ng/mL minaprine, centrifuge 2 min, shake, centrifuge 5 min, inject 50  $\mu$ L aliquot of top organic layer.

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**HPLC VARIABLES****Guard column:** 30  $\times$  4.6 5  $\mu$ m Brownlee cyano spheri-5**Column:** 250  $\times$  4.6 5  $\mu$ m Altex ultrasphere cyano**Mobile phase:** MeCN:THF:water:2 M ammonium formate (pH 4.0) 700:100:195:5**Column temperature:** 20**Flow rate:** 1.5**Injection volume:** 50**Detector:** UV 248

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**CHROMATOGRAM****Retention time:** 7.5**Internal standard:** minaprine (5.5)**Limit of detection:** 20 ng/mL

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**OTHER SUBSTANCES****Simultaneous:** desipramine, clomipramine**Also analyzed:** diltiazem, nortriptyline, amitriptyline, haloperidol, propafenone, amiodarone, verapamil

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**KEY WORDS**serum

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**REFERENCE**Mazzi, G. Simple and practical high-performance liquid chromatographic assay of some tricyclic drugs, haloperidol, diltiazem, verapamil, propafenone, and amiodarone, *Chromatographia*, **1987**, *24*, 313–316.

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**SAMPLE****Matrix:** blood



**Sample preparation:** Inject 200  $\mu$ L serum onto column A and elute with mobile phase A for 10 min then back-flush column A onto column B with mobile phase B for 4 min. Elute column B with mobile phase B and monitor the effluent. Remove column A from circuit and wash with MeCN:water 60:40 for 6 min then with mobile phase A for 10 min.

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#### HPLC VARIABLES

**Column:** A 40  $\times$  4 TSKprecolumn PW (Tosoh); B 150  $\times$  4 TSKgel ODS-80TM (Tosoh)

**Mobile phase:** A 50 mM pH 7.5 potassium phosphate; B MeCN:100 mM pH 2.7 potassium phosphate 32.5:67.5, containing 0.2 g/L sodium 1-heptanesulfonate

**Flow rate:** 1

**Injection volume:** 200

**Detector:** UV 210

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#### CHROMATOGRAM

**Retention time:** 15

**Limit of detection:** 10 ng/mL

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#### OTHER SUBSTANCES

**Simultaneous:** amitriptyline, amoxapine, clomipramine, doxepin, desipramine, maprotiline, trimipramine

**Interfering:** nortriptyline

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#### KEY WORDS

serum; column-switching; use gradient to determine metabolites

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#### REFERENCE

Matsumoto,K.; Kanba,S.; Kubo,H.; Yagi,G.; Iri,H.; Yuki,H. Automated determination of drugs in serum by column-switching high-performance liquid chromatography. IV. Separation of tricyclic and tetracyclic antidepressants and their metabolites, *Clin.Chem.*, **1989**, 35, 453-456.

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#### SAMPLE

**Matrix:** blood

**Sample preparation:** 2 mL Plasma + 100  $\mu$ L 10 mM HCl + 200  $\mu$ L 10% ammonium carbonate (final pH 8.7), vortex gently, add 5 mL MTBE, extract (Vibrax VXR2) for 20 min, centrifuge at 4° at 1720 g for 10 min, remove the organic layer. Add 5 mL dichloromethane to the aqueous layer, shake for 20 min on a reciprocating shaker, centrifuge at 0° at 1720 g for 10 min. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 50°, reconstitute the residue in 100  $\mu$ L 10 mM HCl, wash with 2 mL MTBE, wash with 2 mL hexane, inject a 3-20  $\mu$ L aliquot of the aqueous layer.

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#### HPLC VARIABLES

**Column:** 250  $\times$  4.6 5  $\mu$ m Ultrasphere-ODS C18

**Mobile phase:** MeCN:MeOH:40 mM ammonium acetate 24:40:36 containing 0.04% triethylamine, pH adjusted to 7.3 with glacial acetic acid

**Flow rate:** 1.2

**Injection volume:** 3-20

**Detector:** UV 237

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#### CHROMATOGRAM

**Retention time:** 17.9

**Internal standard:** imipramine

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#### OTHER SUBSTANCES

**Extracted:** diltiazem

**Simultaneous:** alprazolam, amitriptyline, desipramine, loxapine, nortriptyline

**Noninterfering:** clomipramine

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#### KEY WORDS

plasma; imipramine is IS

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**REFERENCE**

Yeung,P.K.F.; Montague,T.J.; Tsui,B.; McGregor,C. High-performance liquid chromatographic assay of diltiazem and six of its metabolites in plasma: application to a pharmacokinetic study in healthy volunteers, *J.Pharm.Sci.*, **1989**, 78, 592-597.

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**SAMPLE**

**Matrix:** blood

**Sample preparation:** 1 mL Plasma + 200  $\mu$ L saturated potassium carbonate, mix, add 5 mL hexane:isopropanol 98:2, shake at 230 rpm for 15 min, centrifuge at 800 g for 10 min. Remove the organic layer and add it to 100  $\mu$ L 0.5% orthophosphoric acid, shake for 15 min, centrifuge at 800 g at 5° for 10 min, inject a 50  $\mu$ L aliquot of the aqueous layer.

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**HPLC VARIABLES**

**Column:** 150  $\times$  4.6 5  $\mu$ m Spherisorb ODS-1

**Mobile phase:** MeCN:water:1 M NaH<sub>2</sub>PO<sub>4</sub> 55:35:10

**Flow rate:** 1.8

**Injection volume:** 50

**Detector:** UV 205

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**CHROMATOGRAM**

**Retention time:** 6.4

**Internal standard:** imipramine

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**OTHER SUBSTANCES**

**Simultaneous:** diphenhydramine

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**KEY WORDS**

plasma; imipramine is IS

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**REFERENCE**

Selinger,K.; Prevost,J.; Hill,H.M. High-performance liquid chromatography method for the determination of diphenhydramine in human plasma, *J.Chromatogr.*, **1990**, 526, 597-602.

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**SAMPLE**

**Matrix:** blood

**Sample preparation:** 1 mL Plasma + 10  $\mu$ L 1 mg/mL 8-hydroxychloroimipramine + 500  $\mu$ L 0.6 M pH 10.4 carbonate buffer + 5 mL ethyl acetate:heptane 20:80, shake for 2.5 min, centrifuge at 3000 g for 10 min. Remove organic layer and add it to 125  $\mu$ L pH 2.4 25 mM KH<sub>2</sub>PO<sub>4</sub>, shake for 2.5 min, centrifuge at 3000 g for 10 min. Remove the aqueous layer and put it in a rotary evaporator for 25 min to remove traces of organic solvent. Inject a 50  $\mu$ L aliquot.

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**HPLC VARIABLES**

**Column:** 120  $\times$  4.6 5  $\mu$ m Nucleosil C18

**Mobile phase:** MeCN:buffer 30:70 (Buffer was 10 Mm KH<sub>2</sub>PO<sub>4</sub> + 5 mM tetramethylammonium chloride adjusted to pH 2.4 with concentrated phosphoric acid.)

**Flow rate:** 1

**Injection volume:** 50

**Detector:** E, ESA Coulochem Model 5100A, detector 1 +0.2 V, detector 2, +0.68 V, guard cell 0.70 V, gain 12  $\times$  10, response time 0.4 s; UV 215

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**CHROMATOGRAM**

**Retention time:** 9.39

**Internal standard:** 8-hydroxychloroimipramine (6.35)

**Limit of detection:** 0.5 ng/mL (electrochemical)

**Limit of quantitation:** 15 ng/mL (UV)

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**OTHER SUBSTANCES**

**Simultaneous:** 2-hydroxydesipramine, 2-hydroxyimipramine, desipramine, chlorodesipramine, chloroimipramine, mesoridazine

**Noninterfering:** doxepin, nordoxepin, amitriptyline, fluoxetine, norfluoxetine, triazolam, alprazolam

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**KEY WORDS**

plasma

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**REFERENCE**

Foglia, J.P.; Sorisio, D.; Perel, J.M. Determination of imipramine, desipramine and their hydroxy metabolites by reversed-phase chromatography with ultraviolet and coulometric detection, *J.Chromatogr.*, **1991**, 572, 247–258.

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**SAMPLE****Matrix:** blood

**Sample preparation:** 1 mL Serum + 3  $\mu$ L 20 ng/mL clobazam + 1 mL saturated sodium borate (adjusted to pH 11 with 6 M NaOH) + 5 mL n-hexane, mix 2 min, centrifuge at 3000 g for 10 min. Remove organic phase and evaporate to dryness under a stream of helium at 30°. Reconstitute in 20  $\mu$ L mobile phase, inject a 10  $\mu$ L aliquot.

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**HPLC VARIABLES****Guard column:** 20 mm long Pelliguard LC-8 40  $\mu$ m (Supelco)**Column:** 150  $\times$  4.6 C8 5  $\mu$ m (Supelco)

**Mobile phase:** MeCN:buffer 50:50 (Buffer was 1.2 mL butylamine in 1 L 10 mM NaH<sub>2</sub>PO<sub>4</sub>, pH adjusted to 3 with phosphoric acid.)

**Flow rate:** 1**Injection volume:** 10**Detector:** UV 254

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**CHROMATOGRAM****Retention time:** k' 3.025**Internal standard:** clobazam (k' 1.344)**Limit of detection:** 10 ng/mL

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**OTHER SUBSTANCES****Extracted:** desipramine, nortriptyline, amitriptyline, clomipramine

**Simultaneous:** nitrazepam, lorazepam, clonazepam, triazolam, flunitrazepam, alprazolam, diazepam, haloperidol, maprotiline

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**KEY WORDS**

serum

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**REFERENCE**

Segatti, M.P.; Nisi, G.; Grossi, F.; Mangiarotti, M.; Lucarelli, C. Rapid and simple high-performance liquid chromatographic determination of tricyclic antidepressants for routine and emergency serum analysis, *J.Chromatogr.*, **1991**, 536, 319–325.

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**SAMPLE****Matrix:** blood

**Sample preparation:** For each 1 mL plasma or serum add 10  $\mu$ L 14  $\mu$ g/mL trimipramine in MeOH. Inject serum or plasma directly onto column A with mobile phase A, elute with mobile phase A to waste. After 15 min elute column A onto column B (foreflush) with mobile phase B. After 2 min remove column A from the circuit, elute column B with mobile phase B, monitor the effluent from column B. Re-equilibrate column A with mobile phase A.

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**HPLC VARIABLES**

**Column:** A 20  $\times$  4.6 10  $\mu$ m Hypersil MOS C8; B 20  $\times$  4.6 5  $\mu$ m Hypersil CPS CN + 250  $\times$  4.6 5  $\mu$ m Nucleosil 100 CN

**Mobile phase:** A MeOH:water 5:95; B MeCN:MeOH:buffer 578:188:235 (Buffer was 10 mM K<sub>2</sub>HPO<sub>4</sub> adjusted to pH 6.8 with 85% phosphoric acid.)

**Flow rate:** 1.5**Injection volume:** 100**Detector:** UV 214

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**CHROMATOGRAM****Retention time:** 9.65

**Internal standard:** trimipramine (6.5)

**Limit of detection:** 1 ng/mL (with three injections onto column A before switching), 5-10 ng/mL

#### OTHER SUBSTANCES

**Extracted:** metabolites, amitriptyline, clomipramine, desipramine, doxepin, fluvoxamine, maprotiline, nortriptyline

**Noninterfering:** chlordiazepoxide, clobazam, clozapine, diazepam, flurazepam, fluspirilene, haloperidol, nitrazepam, oxazepam, perazine, pimozone, spiroperidol, trifluoperidol

#### KEY WORDS

plasma; serum; column-switching

#### REFERENCE

Härtter, S.; Hiemke, C. Column switching and high-performance liquid chromatography in the analysis of amitriptyline, nortriptyline and hydroxylated metabolites in human plasma or serum, *J. Chromatogr.*, **1992**, 578, 273-282.

#### SAMPLE

**Matrix:** blood

**Sample preparation:** Add 10  $\mu$ L 20  $\mu$ g/mL oxaprotiline in MeOH to 990  $\mu$ L plasma or serum. Inject 100  $\mu$ L plasma or serum onto column A with mobile phase A and elute to waste, after 15 min elute column A onto column B with mobile phase B for 2 min. Remove column A from circuit and re-equilibrate it with mobile phase A for 5 min. Chromatograph on column B with mobile phase B.

#### HPLC VARIABLES

**Column:** A 20  $\times$  4.6 10  $\mu$ m Hypersil MOS C8; B 20  $\times$  4.6 5  $\mu$ m Hypersil CPS CN + 250  $\times$  4.6 5  $\mu$ m Nucleosil 100 CN

**Mobile phase:** A MeOH:water 5:95; B MeOH:MeCN:10 mM pH 6.8 potassium phosphate buffer 188:578:235

**Flow rate:** 1.5

**Injection volume:** 100

**Detector:** UV 214

#### CHROMATOGRAM

**Retention time:** 9.7

**Limit of detection:** 20 ng/mL

#### OTHER SUBSTANCES

**Simultaneous:** clozapine, fluvoxamine, metoclopramide, fluoxetine, norfluoxetine, nortriptyline, desipramine, maprotiline, doxepin, clomipramine, amitriptyline

**Noninterfering:** haloperidol, spiroperidol, pimozone, fluspirilene, trifluoperidol, perazine, chlordiazepoxide, clobazam, diazepam, nordiazepam, flurazepam, lorazepam, nitrazepam, oxazepam, carbamazepine

**Interfering:** oxaprotiline

#### KEY WORDS

plasma; serum; column-switching

#### REFERENCE

Härtter, S.; Wetzel, H.; Hiemke, C. Automated determination of fluvoxamine in plasma by column-switching high-performance liquid chromatography, *Clin. Chem.*, **1992**, 38, 2082-2086.

#### SAMPLE

**Matrix:** blood

**Sample preparation:** Condition a 1 mL BondElut C18 SPE cartridge with 1 mL 1 M HCl, 1 mL MeOH, 1 mL water, and 1 mL 1% potassium carbonate. 700  $\mu$ L Serum + 50  $\mu$ L 5  $\mu$ g/mL trimipramine in 5% potassium bicarbonate + 700  $\mu$ L MeCN, vortex, centrifuge at 1500 g for 5 min, add supernatant to SPE cartridge (at ca. 1 mL/min). Wash with 2 mL water and 1 mL MeCN, elute with 250  $\mu$ L MeOH:35% perchloric acid 20:1 by gravity (10 min) then centrifuge for 20 s to remove rest of eluant, inject a 50  $\mu$ L aliquot of the eluate.

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**HPLC VARIABLES****Guard column:** 15 mm 7  $\mu$ m Brownlee RP-8**Column:** 150  $\times$  4.6 5  $\mu$ m Ultrasphere Octyl**Mobile phase:** MeCN:water 37.5:62.5 containing 0.5 g/L tetramethylammonium perchlorate and 0.5 mL/L 7% perchloric acid**Flow rate:** 1.5**Injection volume:** 50**Detector:** UV 215

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**CHROMATOGRAM****Retention time:** 7.1**Internal standard:** trimipramine (9.6)**Limit of quantitation:** 5 ng/mL

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**OTHER SUBSTANCES****Extracted:** amitriptyline, clomipramine, desipramine, doxepin, fluoxetine, maprotiline, protriptyline**Interfering:** desmethylmaprotiline, fluvoxamine, nortriptyline

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**KEY WORDS**serum; SPE

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**REFERENCE**Gupta, R.N. An improved solid phase extraction procedure for the determination of antidepressants in serum by column liquid chromatography, *J.Liq.Chromatogr.*, **1993**, 16, 2751-2765.

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**SAMPLE****Matrix:** blood**Sample preparation:** 1 mL Plasma + 100  $\mu$ L 200 ng/mL IS in MeOH + 1 mL 50 mM pH 10 borate buffer, vortex briefly, add to an Extrelut 3 SPE cartridge, let stand for 5 min, elute with 15 mL hexane:dichloromethane 50:50. Add the eluate to 3 mL 50 mM sulfuric acid, mix for 10 min, centrifuge at 3000 g for 10 min. Remove the aqueous layer and add it to 6 mL hexane:dichloromethane 50:50, wash for 5 min, centrifuge. Make the aqueous layer basic with 150  $\mu$ L 28% ammonia, extract twice with 3 mL hexane:dichloromethane 50:50. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 60°, reconstitute the residue in 100  $\mu$ L mobile phase, inject a 20  $\mu$ L aliquot.

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**HPLC VARIABLES****Guard column:** 30  $\times$  4.6 5  $\mu$ m Spherisorb cyano**Column:** 250  $\times$  4.6 5  $\mu$ m Ultrasphere cyano**Mobile phase:** MeCN:buffer 60:40 (Buffer was 50 mM  $\text{KH}_2\text{PO}_4$  adjusted to pH 6.5 with 28% ammonia.)**Flow rate:** 1**Injection volume:** 20**Detector:** E, 5100 A Coulochem, 5020 guard cell 1.00 V, 5011 analytical cell, detector 1 0.55 V, detector 2 0.80 V, output of detector 2 is monitored

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**CHROMATOGRAM****Retention time:** 19.7**Internal standard:** methylrisperidone (R68808) (14.3)

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**OTHER SUBSTANCES****Extracted:** chlorpromazine, clomipramine, cyamemazine, desipramine, droperidol, flunitrazepam, haloperidol, pipamperone, risperidone**Noninterfering:** alprazolam, bromazepam, carbamazepine, chlorazepate, diazepam, diphenylhydantoin, estazolam, ethylbenzotropine, oxazepam, phenobarbital, triazolam, valproic acid**Interfering:** trihexyphenidyl

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**KEY WORDS**

plasma; SPE

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**REFERENCE**

Le Moing,J.P.; Edouard,S.; Levron,J.C. Determination of risperidone and 9-hydroxyrisperidone in human plasma by high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.*, **1993**, *614*, 333–339.

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**SAMPLE**

**Matrix:** blood

**Sample preparation:** Automated SPE by ASPEC system. Condition a C18 Clean-Up SPE cartridge (CEC 18111, Worldwide Monitoring) with 2 mL MeOH then 2 mL water. 1 mL Plasma + 1 mL 400 ng/mL protriptyline in water, vortex, add to column, wash with 3 mL water, wash with 3 mL 750 mL/L methanol. Elute with three aliquots of 300  $\mu$ L 0.1 M ammonium acetate in MeOH. Add 0.5 mL 0.5 M NaOH and 4 mL 50 mL/L isopropanol in heptane to eluate, mix thoroughly. Allow 5 min for phase separation. Remove upper heptane phase and add it to 300  $\mu$ L 0.1 M phosphoric acid (pH 2.5), mix, separate, inject a 100  $\mu$ L aliquot of the aqueous phase.

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**HPLC VARIABLES**

**Guard column:** LC-8-DB (Supelco)

**Column:** 150  $\times$  4.6 LC-8-DB (Supelco)

**Mobile phase:** MeCN:buffer 35:65 (Buffer was 10 mL/L triethylamine in water adjusted to pH 5.5 with glacial acetic acid.)

**Flow rate:** 2

**Injection volume:** 100

**Detector:** UV 228

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**CHROMATOGRAM**

**Retention time:** 4.6

**Internal standard:** protriptyline (4)

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**OTHER SUBSTANCES**

**Extracted:** acetazolamide, amitriptyline, chlordiazepoxide, chlorimipramine, chlorpromazine, desipramine, dextromethorphan, diazepam, diphenhydramine, doxepin, encainide, fentanyl, flecainide, fluoxetine, flurazepam, haloperidol, hydroxyethylflurazepam, ibuprofen, lidocaine, methadone, methaqualone, mexiletine, midazolam, norchlorimipramine, nordiazepam, nordoxepin, norfluoxetine, norverapamil, pentazocine, promazine, propafenone, propoxyphene, propranolol, protriptyline, quinidine, temazepam, trazodone, trimipramine

**Noninterfering:** acetaminophen, acetylmorphine, amiodarone, amobarbital, amphetamine, benodroflumethiazide, benzocaine, benzoylecgonine, benzthiazide, butalbital, carbamazepine, chlorothiazide, clonazepam, cocaine, codeine, cotinine, cyclosporine, cyclothiazide, desalkylflurazepam, diamorphine, dicumerol, ephedrine, ethacrynic acid, ethanol, ethchlorvynol, ethosuximide, furosemide, glutethimide, hydrochlorothiazide, hydrocodone, hydroflumethiazide, hydromorphone, lorazepam, mephentermine, meprobamate, methamphetamine, metharbital, methoxsalen, methoxyphenteramine, methsuximide, methylcyclothiazide, metoprolol, MHPG, monoacetylmorphine, morphine, normethsuximide, oxazepam, oxycodone, oxymorphone, pentobarbital, phenacyclidine, phenteramine, phenylephrine, phenytoin, polythiazide, primidone, prochlorperazine, salicylic acid, sulfanilamide, THC-COOH, theophylline, thiazolam, thiopental, thioridazine, tocinide, trichloromethiazide, trifluoperazine, valproic acid, warfarin

**Interfering:** maprotiline, nortriptyline, verapamil

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**KEY WORDS**

plasma; SPE

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**REFERENCE**

Nichols,J.H.; Charlson,J.R.; Lawson,G.M. Automated HPLC assay of fluoxetine and norfluoxetine in serum, *Clin.Chem.*, **1994**, *40*, 1312–1316.

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**SAMPLE**

**Matrix:** blood

**Sample preparation:** 500  $\mu$ L Plasma + 100  $\mu$ L EtOH + 500  $\mu$ L 500 mM NaOH, mix, add to an Extrelut-1 SPE cartridge, let stand for 10 min, elute with 5 mL n-hexane:isoamyl alcohol 98:2. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 100  $\mu$ L mobile phase, inject an aliquot.

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**HPLC VARIABLES**

**Column:** 125 × 4.6 5 µm Partisphere silica (Whatman)

**Mobile phase:** Hexane:EtOH:dichloromethane:diethylamine 77:18:5:0.003

**Flow rate:** 1.3

**Detector:** UV 214

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**CHROMATOGRAM**

**Retention time:** 3

**Internal standard:** imipramine

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**OTHER SUBSTANCES**

**Extracted:** clomipramine

**Simultaneous:** amitriptyline, desipramine, doxepin, fluoxetine

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**KEY WORDS**

plasma; SPE; normal phase; imipramine is IS

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**REFERENCE**

Altieri,I.; Pichini,S.; Pacifici,R.; Zuccaro,P. Improved clean-up procedure for the high-performance liquid chromatographic assay of clomipramine and its demethylated metabolite in human plasma, *J.Chromatogr.B*, **1995**, 669, 416-417.

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**SAMPLE**

**Matrix:** blood

**Sample preparation:** 1 mL Plasma + 1 mL 500 mM pH 9 phosphate buffer, vortex briefly, add 7 mL n-heptane:isoamyl alcohol 98.5:1.5, shake on a rotating shaker at 32 rpm for 15 min, centrifuge at 1500 g for 5 min. Remove 6 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 60°, reconstitute the residue in 200 µL mobile phase, vortex for 10 s, centrifuge at 1500 g for 3 min, inject a 100 µL aliquot of the supernatant.

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**HPLC VARIABLES**

**Guard column:** 20 × 4.6 5 µm Supelguard LC-8-DB (Supelco)

**Column:** 150 × 4.6 5 µm Supelcosil LC-8-DB

**Mobile phase:** MeCN:MeOH:buffer 20:25:55 (Buffer was 50 mM pH 3.0 KH<sub>2</sub>PO<sub>4</sub> containing 0.2% triethylamine.)

**Flow rate:** 1

**Injection volume:** 100

**Detector:** UV 235

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**CHROMATOGRAM**

**Retention time:** 11.6

**Internal standard:** imipramine

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**OTHER SUBSTANCES**

**Extracted:** ticlopidine

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**KEY WORDS**

plasma; imipramine is IS

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**REFERENCE**

Dal Bo,L.; Verga,F.; Marzo,A.; Ambrosoli,L.; Poli,A. Determination of ticlopidine in human plasma by high-performance liquid chromatography and ultraviolet absorbance detection, *J.Chromatogr.B*, **1995**, 665, 404-409.

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**SAMPLE**

**Matrix:** blood

**Sample preparation:** 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of

the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

#### HPLC VARIABLES

**Column:** 300 × 3.9 4 µm NovaPack C18

**Mobile phase:** MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH<sub>2</sub>PO<sub>4</sub> adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)

**Column temperature:** 30

**Flow rate:** 0.8

**Injection volume:** 50

**Detector:** UV 251

#### CHROMATOGRAM

**Retention time:** 8.53

**Limit of detection:** <120 ng/mL

#### KEY WORDS

whole blood; plasma; interferences may occur—compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; car-teolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihy-dralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazo-lam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lor-azepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbin; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temaze-pam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; almino-profen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acen-ocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; fle-cainide; phenacyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; bupren-orphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazo-lam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; aceprometazine; glibenclam-ide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluoperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; metha-done; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidra-mine; proprietyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvox-amine; pimozone; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tiocloamarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpi-pramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

#### REFERENCE

Tracqui, A.; Kintz, P.; Mangin, P. Systematic toxicological analysis using HPLC/DAD, *J. Forensic Sci.*, **1995**, *40*, 254–262.

#### SAMPLE

**Matrix:** blood

**Sample preparation:** 100 µL Serum + 25 µL 5 µg/mL clomipramine in MeOH, vortex for 30 s, add 100 µL 5 M NaOH, add 2 mL hexane, vortex for 30 s, centrifuge at 3000 g for 3 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 20°, re-constitute the residue in 50 µL mobile phase, vortex for 30 s, inject a 20 µL aliquot.



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**HPLC VARIABLES**

**Column:** 150 × 4.6 5 µm Microsorb MV octadecyl

**Mobile phase:** MeCN:10 mM triethylamine 60:40, pH adjusted to 3.0 with 85% phosphoric acid

**Flow rate:** 1

**Injection volume:** 20

**Detector:** UV 260

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**CHROMATOGRAM**

**Retention time:** 6.0

**Internal standard:** clomipramine (8.3)

**Limit of quantitation:** 25 ng/mL

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**OTHER SUBSTANCES**

**Extracted:** desipramine

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**KEY WORDS**

mouse; serum; pharmacokinetics

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**REFERENCE**

Yoo,S.D.; Holladay,J.W.; Fincher,T.K.; Dewey,M.J. Rapid microsample analysis of imipramine and desipramine by reversed-phase high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.B*, 1995, 668, 338–342.

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**SAMPLE**

**Matrix:** blood, CSF, tissue

**Sample preparation:** Homogenize brain tissue in 4 mL 50 mM pH 7.4 Tris-HCl. 500 µL Serum or 200 µL CSF or 1 mL homogenate + 100 µL 2 M NaOH + IS + 5 mL hexane:dichloromethane 60:40, shake for 15 min, centrifuge at 4000 rpm for 10 min. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 100 µL mobile phase, inject a 30 µL aliquot.

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**HPLC VARIABLES**

**Column:** 125 × 4 5 µm Lichrospher RP select B

**Mobile phase:** MeCN:0.1% pH 4 diethylamine in water 40:60

**Flow rate:** 1.5

**Injection volume:** 30

**Detector:** UV 254

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**CHROMATOGRAM**

**Retention time:** 13.85

**Internal standard:** desmethylclomipramine chlorhydrate (6.85)

**Limit of detection:** 25 ng/mL

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**OTHER SUBSTANCES**

**Extracted:** metabolites, desipramine

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**KEY WORDS**

rat; serum; brain; pharmacokinetics

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**REFERENCE**

Besret,L.; Debruyne,D.; Rioux,P.; Bonvalot,T.; Moulin,M.; Zarifian,E.; Baron,J.-C. A comprehensive investigation of plasma and brain regional pharmacokinetics of imipramine and its metabolites during and after chronic administration in the rat, *J.Pharm.Sci.*, 1996, 85, 291–295.

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**SAMPLE**

**Matrix:** blood, tissue

**Sample preparation:** Serum. 1 mL Serum + 800 ng nortriptyline + 4 mL MeOH + 5 mL 2.5% perchloric acid, shake vigorously, centrifuge at 11000 g for 15 min. Add supernatant to 1 mL 4 M KOH, centrifuge. Add supernatant (9 mL) to 10 mL diethyl ether:ethyl acetate 85:15, shake for 15 min. Remove 8 mL of organic phase and evaporate it to dryness under a stream of nitrogen. Dissolve residue in 200 µL mobile phase buffer:MeOH 9:1, inject 100 µL aliquot. Tissue. 2 g Brain tissue + 10 mL 2.5% perchloric acid + 8 mL MeOH + 1.6 µg nortriptyline,

homogenize, centrifuge at 11000 g for 15 min. Add supernatant to 4 mL 4 M KOH, centrifuge. Add supernatant to 20 mL diethyl ether:ethyl acetate 3:1, shake for 15 min. Remove 8 mL of organic phase and evaporate it to dryness under a stream of nitrogen. Dissolve residue in 200  $\mu$ L mobile phase buffer:MeOH 9:1, inject 100  $\mu$ L aliquot.

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**HPLC VARIABLES**

**Column:** 250  $\times$  4.6 5  $\mu$ m Cosmosil 5C18

**Mobile phase:** MeOH:THF:buffer 45:17:88 (Buffer was 1% triethylamine adjusted to pH 3.0 with phosphoric acid.)

**Column temperature:** 40

**Flow rate:** 1

**Injection volume:** 100

**Detector:** UV 254

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**CHROMATOGRAM**

**Retention time:** 11.2

**Internal standard:** nortriptyline (14.2)

**Limit of detection:** 10 ng/g (tissue)

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**OTHER SUBSTANCES**

**Simultaneous:** desipramine

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**KEY WORDS**

serum; rat

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**REFERENCE**

Sugita,S.; Kobayashi,A.; Suzuki,S.; Yoshida,T.; Nakazawa,K. High-performance liquid chromatographic determination of imipramine and its metabolites in rat brain, *J.Chromatogr.*, **1987**, *421*, 412-417.

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**SAMPLE**

**Matrix:** blood, tissue

**Sample preparation:** Blood or serum. 1 mL Blood or serum + 1  $\mu$ g cianopramine + 1 mL water, vortex, add 1 mL 200 mM sodium carbonate, vortex, add 6 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 100  $\mu$ L 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30  $\mu$ L aliquot of the aqueous layer. Liver homogenate. 0.5 mL Liver homogenate + 10  $\mu$ g cianopramine + 500  $\mu$ L 2% sodium tetraborate + 8 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 400  $\mu$ L 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30  $\mu$ L aliquot of the aqueous layer.

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**HPLC VARIABLES**

**Guard column:** 15  $\times$  3.2 7  $\mu$ m RP-18 Newguard (Applied Biosystems)

**Column:** 100  $\times$  4.6 5  $\mu$ m Brownlee Spheri-5 RP-18

**Mobile phase:** MeCN:100 mM NaH<sub>2</sub>PO<sub>4</sub>:diethylamine 40:57.5:2.5

**Flow rate:** 2

**Injection volume:** 30

**Detector:** UV 220

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**CHROMATOGRAM**

**Retention time:** 17.9

**Internal standard:** cianopramine (8.93)

**Limit of detection:** 50 ng/mL

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**OTHER SUBSTANCES**

**Simultaneous:** amitriptyline, amoxapine, benztropine, brompheniramine, chlorpheniramine, chlorpromazine, clomipramine, cyproheptadine, desipramine, diphenhydramine, dothiepin, doxepin, fluoxetine, haloperidol, loxapine, maprotiline, meperidine, mesoridazine, methadone, metoclopramide, mianserin, moclobemide, nomifensine, nordoxepin, norfluoxetine, norpropoxyphene, nortriaden, nortriptyline, pentobarbital, pheniramine, promethazine, propoxyphene, propranolol, protriptyline, quinidine, quinine, sulforidazine, thioridazine, tranlycypromine, trazodone, trihexphenidyl, trimipramine, triprolidine

**Noninterfering:** dextromethorphan, norphethidine, phenoxybenzamine, prochlorperazine, trifluoperazine

**Interfering:** thiothixene

## KEY WORDS

serum; whole blood; liver

## REFERENCE

McIntyre, I.M.; King, C.V.; Skafidis, S.; Drummer, O.H. Dual ultraviolet wavelength high-performance liquid chromatographic method for the forensic or clinical analysis of seventeen antidepressants and some selected metabolites, *J.Chromatogr.*, **1993**, 621, 215–223.

## SAMPLE

**Matrix:** blood, tissue, urine

**Sample preparation:** Serum, urine. 500  $\mu$ L Serum or urine + 100  $\mu$ L 2  $\mu$ g/mL diazepam + 200  $\mu$ L 20% sodium carbonate + 500  $\mu$ L water + 3 mL n-hexane:isoamyl alcohol 98.5:1.5, mix for 2 min, centrifuge at 1200 g for 5 min. Remove the organic phase and evaporate it under a gentle stream of nitrogen at about 40°. Dissolve the residue in 100  $\mu$ L mobile phase, inject a 10  $\mu$ L aliquot. Tissue. Homogenize 1 g sample with 9 mL 100 mM HCl and 100  $\mu$ L 20  $\mu$ g/mL diazepam, centrifuge at 15000 g for 10 min. Add 500  $\mu$ L 20% sodium carbonate and 4 mL n-hexane:isoamyl alcohol 98.5:1.5 to 1 mL of the supernatant, mix for 5 min. Remove the organic phase and evaporate it under a gentle stream of nitrogen at about 40°. Dissolve the residue in 100  $\mu$ L mobile phase, filter by microconcentrator (Microcon-30, Grace). Inject a 10  $\mu$ L aliquot.

## HPLC VARIABLES

**Column:** 100  $\times$  4.6 2  $\mu$ m TSK gel Super-Octyl (A) or 100  $\times$  4.6 5  $\mu$ m Hypersil MOS-C8 (B), (Yokogawa, Japan)

**Mobile phase:** MeOH:20 mM pH 7  $\text{KH}_2\text{PO}_4$  60:40

**Flow rate:** 0.6

**Injection volume:** 10

**Detector:** UV 254

## CHROMATOGRAM

**Retention time:** 12.8 (A), 23.8 (B)

**Internal standard:** diazepam (4.4, A)

**Limit of quantitation:** 50 ng/mL (serum, urine) (A), 500 ng/mL (tissue) (A)

## OTHER SUBSTANCES

**Extracted:** amitriptyline, amoxapine, clomipramine, desipramine, dothiepin, doxepin, maprotiline, melitracen, mianserin, nortriptyline

**Noninterfering:** barbitol, carbamazepine, ethosuximide, hexobarbital, lofepramine, pentobarbital, phenobarbital, phenytoin, primidone, sulpiride, trimethadione, trimipramine

## KEY WORDS

serum; brain; liver

## REFERENCE

Tanaka, E.; Terada, M.; Nakamura, T.; Misawa, S.; Wakasugi, C. Forensic analysis of eleven cyclic antidepressants in human biological samples using a new reversed-phase chromatographic column of 2  $\mu$ m porous microspherical silica gel, *J.Chromatogr.B*, **1997**, 692, 405–412.

## SAMPLE

**Matrix:** blood, urine

**Sample preparation:** 1 mL Plasma or urine + 20  $\mu$ L 10  $\mu$ g/mL pericyazine in MeOH, mix, add 0.2 mL 1 M sodium carbonate buffer to adjust pH to 9.6. Add 10 mL distilled diethyl ether, shake on an automatic shaker for 10 min, centrifuge at 1000 g for 10 min in a refrigerated centrifuge. Remove the upper organic layer and add 100  $\mu$ L 100 mM orthophosphoric acid. Shake for 10 min and centrifuge at 1000 g for 10 min. Discard the top layer and inject a 50  $\mu$ L aliquot of the acid layer.

## HPLC VARIABLES

**Guard column:** 40  $\times$  4.6 10  $\mu$ m RP-18

**Column:** 300 × 3.9 Bondclone 10 C18 (Phenomenex)

**Mobile phase:** MeCN:100 mM K<sub>2</sub>HPO<sub>4</sub>, adjust pH to 6.0 with orthophosphoric acid

**Flow rate:** 2

**Injection volume:** 50

**Detector:** E, EDT Chromajet, oxidation cell +1.00 V

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#### CHROMATOGRAM

**Retention time:** 12.3

**Internal standard:** pericyazine (6.2)

**Limit of detection:** 3 ng/mL

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#### OTHER SUBSTANCES

**Extracted:** metabolites, desipramine

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#### KEY WORDS

plasma; pharmacokinetics; silanize glassware

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#### REFERENCE

Chen, A.G.; Wing, Y.K.; Chiu, H.; Lee, S.; Chen, C.N.; Chan, K. Simultaneous determination of imipramine, desipramine and their 2- and 10-hydroxylated metabolites in human plasma and urine by high-performance liquid chromatography, *J. Chromatogr. B*, **1997**, 693, 153–158.

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#### SAMPLE

**Matrix:** blood, urine

**Sample preparation:** 1 mL Plasma or urine + 1 mL 600 mM pH 11.3 K<sub>2</sub>CO<sub>3</sub> + 100 µL 5 (plasma) or 20 (urine) µM 2-hydroxydesmethyldomipramine in EtOH + 5 mL heptane:MTBE 1:1 + 5% n-butanol, vortex 1 min, centrifuge at 1400 g for 10 min, freeze at -50° (dry ice/ethanol). Remove organic layer and add it to 1 mL 20 mM HCl, vortex 1 min, centrifuge at 1400 g for 10 min, freeze at -50° (dry ice/ethanol). Discard organic layer. Thaw out aqueous layer and make alkaline (pH 11) by adding 500 µL 600 mM pH 11.3 K<sub>2</sub>CO<sub>3</sub>. Add 3 mL heptane:MTBE 1:1 + 5% n-butanol, vortex 1 min, centrifuge at 1400 g for 10 min, freeze at -50° (dry ice/ethanol). Remove organic layer and evaporate it to dryness at 50° under a stream of nitrogen. Dissolve residue in 100 µL mobile phase, vortex for 5 s, centrifuge at 1400 g for 1 min, inject a 20 µL aliquot.

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#### HPLC VARIABLES

**Guard column:** 20 × 4 7 µm 120 Å Nucleosil

**Column:** 250 × 4 5 µm 100 Å Nucleosil RP-phenyl

**Mobile phase:** MeCN:buffer 30:70 (Buffer was 14.05 g sodium perchlorate and 1.6 mL 60% perchloric acid in 5 L water, pH 2.5.)

**Column temperature:** 30

**Flow rate:** 1

**Injection volume:** 20

**Detector:** UV 220

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#### CHROMATOGRAM

**Retention time:** 16.62

**Internal standard:** 2-hydroxydesmethyldomipramine (10.02)

**Limit of detection:** 10 nM (urine), 5 nM (plasma)

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#### OTHER SUBSTANCES

**Simultaneous:** desipramine, metabolites

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#### KEY WORDS

plasma

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#### REFERENCE

Nielsen, K.K.; Brsen, K. High-performance liquid chromatography of imipramine and six metabolites in human plasma and urine, *J. Chromatogr.*, **1993**, 612, 87–94.

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#### SAMPLE

**Matrix:** blood, urine

**Sample preparation:** 100  $\mu$ L Serum or urine + 25  $\mu$ L 5  $\mu$ g/mL clomipramine in MeOH + 100  $\mu$ L 5 M NaOH + 2 mL hexane, vortex for 30 s, centrifuge at 5000 rpm for 3 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 20°, reconstitute the residue in 50  $\mu$ L mobile phase, inject a 20  $\mu$ L aliquot.

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#### HPLC VARIABLES

**Column:** 10  $\times$  4.6 5  $\mu$ m Microsorb MV C18

**Mobile phase:** MeCN:10 mM triethylamine in water 60:40, pH adjusted to 3.0 with 85% phosphoric acid

**Flow rate:** 1

**Injection volume:** 20

**Detector:** UV 260

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#### CHROMATOGRAM

**Internal standard:** clomipramine

**Limit of quantitation:** 10 ng/mL

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#### OTHER SUBSTANCES

**Extracted:** desipramine

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#### KEY WORDS

mouse; serum; pharmacokinetics

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#### REFERENCE

Yoo,S.D.; Holladay,J.W.; Fincher,T.K.; Baumann,H.; Dewey,M.J. Altered disposition and antidepressant activity of imipramine in transgenic mice with elevated  $\alpha$ -1-acid glycoprotein, *J.Pharmacol.Exp.Ther.*, **1996**, 276, 918–922.

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#### SAMPLE

**Matrix:** blood, urine

**Sample preparation:** Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50  $\mu$ L MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood)  $\mu$ L aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

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#### HPLC VARIABLES

**Guard column:** 20 mm long Symmetry C18

**Column:** 250  $\times$  4.6 5  $\mu$ m Symmetry C8 (Waters)

**Mobile phase:** Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

**Column temperature:** 30

**Flow rate:** 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

**Injection volume:** 10-30

**Detector:** UV 200.5

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#### CHROMATOGRAM

**Retention time:** 15.113

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#### KEY WORDS

whole blood

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#### REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, 763, 149–163.

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**SAMPLE**

**Matrix:** formulations

**Sample preparation:** Crush tablet or capsule, to 2 mg amitriptyline add 20 mL MeOH, shake 30 min, centrifuge at 2000 rpm for 5 min, to 5 mL supernatant add 4 mL 1.25 mg/mL norephedrine.HCl in MeOH, dilute to 10 mL with MeOH.

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**HPLC VARIABLES**

**Column:** 150 × 4.6 5 µm Zorbax CN

**Mobile phase:** MeCN:MeOH:25 mM pH 4.8 sodium acetate-acetic acid buffer 35:45:20

**Flow rate:** 2.5

**Injection volume:** 10

**Detector:** UV 254

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**CHROMATOGRAM**

**Retention time:** 3.8

**Internal standard:** norephedrine (2.7)

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**OTHER SUBSTANCES**

**Also analyzed:** chlorpromazine, amitriptyline, thioridazine, trifluoperazine

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**KEY WORDS**

tablets; capsules

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**REFERENCE**

Lovering,E.G.; Beaulieu,N.; Lawrence,R.C.; Sears,R.W. Liquid chromatographic method for identity, assay, and content uniformity of five tricyclic drugs, *J.Assoc.Off.Anal.Chem.*, **1985**, 68, 168–171.

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**SAMPLE**

**Matrix:** hair

**Sample preparation:** Wash hair in water, rinse 3 times with MeOH, dry, weigh. 5-25 mg Washed hair + 1 mL 1 M NaOH, heat at 70° for 30 min, adjust pH to 9.5-10. 1 mL Extract + 1 µg protriptyline + 1 mL water + 1 mL 200 mM sodium carbonate buffer, mix, extract with hexane: butanol 95:5 for 20 min. Remove the organic layer and add it to 100 µL 0.2% orthophosphoric acid, mix for 20 min, inject a 30 µL aliquot of the aqueous layer.

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**HPLC VARIABLES**

**Guard column:** 15 × 3.2 7 µm Newguard RP-18

**Column:** 100 × 4.6 Spheri-5 RP-C18

**Mobile phase:** MeCN:buffer 40:60 (Buffer was 1.2 L 100 mM pH 7.0 NaH<sub>2</sub>PO<sub>4</sub> + 30 mL diethylamine.)

**Flow rate:** 2

**Injection volume:** 30

**Detector:** UV 214

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**CHROMATOGRAM**

**Internal standard:** protriptyline (4)

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**OTHER SUBSTANCES**

**Extracted:** amitriptyline, clomipramine, desipramine, dothiepin, doxepin, haloperidol, mianserin, nortriptyline

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**KEY WORDS**

may be interferences

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**REFERENCE**

Couper,F.J.; McIntyre,I.M.; Drummer,O.H. Extraction of psychotropic drugs from human scalp hair, *J.Forensic Sci.*, **1995**, 40, 83–86.

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**SAMPLE**

**Matrix:** microsomal incubations

**Sample preparation:** Add 200  $\mu$ L MeCN to 200  $\mu$ L microsomal incubation, centrifuge at 3000 g for 5 min, inject an aliquot of the supernatant.

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#### HPLC VARIABLES

**Column:** 150  $\times$  4.6 Zorbax Phenyl

**Mobile phase:** MeCN:buffer 50:50 (Buffer was 20 mM perchloric acid adjusted to pH 2.5 with NaOH.)

**Flow rate:** 1

**Injection volume:** 120

**Detector:** Radioactivity, Inus  $\beta$ -Ram using Inus Tru-Count scintillation fluid at a flow rate of 5 mL/min

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#### CHROMATOGRAM

**Retention time:** 4.7

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#### OTHER SUBSTANCES

**Extracted:** metabolites

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#### KEY WORDS

human; liver

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#### REFERENCE

Obach,R.S. Nonspecific binding to microsomes: Impact on scale-up of in vitro intrinsic clearance to hepatic clearance as assessed through examination of warfarin, imipramine, and propranolol, *Drug Metab.Dispos.*, **1997**, 25, 1359–1369.

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#### SAMPLE

**Matrix:** microsomal incubations

**Sample preparation:** Condition a C18 SepPak SPE cartridge with 5 mL MeOH and 5 mL buffer. 1 mL Microsomal incubation + 500  $\mu$ L buffer, mix, add a 500  $\mu$ L aliquot to the SPE cartridge, wash with 4 mL MeOH:buffer containing 0.01% ascorbic acid 5:95, elute with 5 mL MeOH, evaporate eluate to dryness, reconstitute in mobile phase, inject an aliquot. (Buffer was 100 mM pH 3.0 potassium acetate buffer containing 5 mM n-heptanesulfonic acid.)

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#### HPLC VARIABLES

**Guard column:** used but not specified

**Column:** 100  $\times$  8 4  $\mu$ m Nova-Pak C18

**Mobile phase:** Gradient. MeOH:MeCN:buffer 20:35:45 for 20 min, then 30:50:20 for 25 min, then 35:60:5

**Flow rate:** 0.4

**Injection volume:** 50-300

**Detector:** UV 254

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#### CHROMATOGRAM

**Retention time:** 33

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#### OTHER SUBSTANCES

**Extracted:** metabolites, desipramine, lofepramine

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#### KEY WORDS

rat; human; liver; SPE

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#### REFERENCE

Strandgärden,K.; Gunnarsson,P.O. Metabolism of lofepramine and imipramine in liver microsomes from rat and man, *Xenobiotica*, **1994**, 24, 703–711.

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#### SAMPLE

**Matrix:** microsomal incubations

**Sample preparation:** 1 mL Microsomal incubation + 500  $\mu$ L 2 M pH 12 sodium carbonate + 5 mL ether, vortex, centrifuge at 1000 g for 10 min. Remove the organic layer and add it to 1

mL 100 mM HCl, vortex, centrifuge. Remove the aqueous layer and add it to 100  $\mu$ L 2 M pH 12 sodium carbonate and 1 mL ether, extract. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100  $\mu$ L mobile phase, inject a 50  $\mu$ L aliquot.

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**HPLC VARIABLES**

**Column:** 250  $\times$  4.6 5  $\mu$ m Supelcosil LC-PCN

**Mobile phase:** MeCN:MeOH:10 mM pH 7  $K_2HPO_4$  40:35:25

**Flow rate:** 1.4

**Injection volume:** 50

**Detector:** UV 214

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**CHROMATOGRAM**

**Retention time:** 7.83

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**OTHER SUBSTANCES**

**Extracted:** metabolites, desipramine

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**KEY WORDS**

rat; liver; brain

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**REFERENCE**

Sequeira,D.J.; Strobel,H.W. High-performance liquid chromatographic method for the analysis of imipramine metabolism in vitro by liver and brain microsomes, *J.Chromatogr.B*, **1995**, 673, 251–258.

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**SAMPLE**

**Matrix:** solutions

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**HPLC VARIABLES**

**Column:** 250  $\times$  4 ODS (Hitachi)

**Mobile phase:** MeCN:50 mM phosphoric acid 50:50 containing 150 mM KCl

**Column temperature:** 55

**Flow rate:** 0.6

**Injection volume:** 20

**Detector:** UV 251

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**REFERENCE**

Sugawara,M.; Takekuma,Y; Yamada,H.; Kobayashi,M.; Iseki,K.; Miyazaki,K. A general approach for the prediction of the intestinal absorption of drugs: regression analysis using the physicochemical properties and drug-membrane electrostatic interactions, *J.Pharm.Sci.*, **1998**, 87, 960–966.

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**SAMPLE**

**Matrix:** solutions

**Sample preparation:** Prepare a 10  $\mu$ g/mL solution in MeOH, inject a 20  $\mu$ L aliquot.

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**HPLC VARIABLES**

**Column:** 125  $\times$  4.9 Spherisorb S5W silica

**Mobile phase:** MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7

**Flow rate:** 2

**Injection volume:** 20

**Detector:** E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

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**CHROMATOGRAM**

**Retention time:** 4.5



## OTHER SUBSTANCES

**Also analyzed:** acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bupofenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipiprone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserine, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamlamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, pimindine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranlycypromine, trazodone, trifluoperazine, trifluoperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripeleminamine, triprolidine, tryptamine, verapamil, xylometazoline

## REFERENCE

Jane, I.; McKinnon, A.; Flanagan, R. J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J. Chromatogr.*, **1985**, *323*, 191–225.

## SAMPLE

**Matrix:** solutions

**Sample preparation:** Dissolve in MeOH:water 1:1 at a concentration of 50 µg/mL, inject a 10 µL aliquot.

## HPLC VARIABLES

**Column:** 300 × 3.9 10 µm µBondapak C18

**Mobile phase:** MeOH:acetic acid:triethylamine:water 60:1.5:0.5:38

**Flow rate:** 1.5

**Injection volume:** 10

**Detector:** UV

## CHROMATOGRAM

**Retention time:** k' 2.14

## REFERENCE

Roos, R. W.; Lau-Cam, C. A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J. Chromatogr.*, **1986**, *370*, 403–418.

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**SAMPLE****Matrix:** solutions**Sample preparation:** Prepare a 500 µg/mL solution in MeOH:water 50:50, inject a 5 µL aliquot.

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**HPLC VARIABLES****Column:** 250 × 4.6 Zorbax C8**Mobile phase:** Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L MeCN:water 20:80. A:B from 100:0 to 0:100 over 30 min. (Purify triethylamine as follows. Wash neutral alumina (Merck) 3 times with 2 bed volumes of pentane, 3 times with 2 bed volumes of dichloromethane, and 3 times with 2 bed volumes of MeOH, allow solvent to evaporate in a fume hood overnight, heat alumina at 130° for 2 h. Prepare a 14 cm column of the washed alumina in a 290 × 22 tube, pass through a head volume of MeOH, pass through triethylamine. When triethylamine starts to elute discard the first 20 mL, use the next 20 mL, discard the column.)**Flow rate:** 2**Injection volume:** 5**Detector:** UV 210

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**CHROMATOGRAM****Retention time:** 18.7

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**OTHER SUBSTANCES****Simultaneous:** acetophenone, amphetamine, desipramine, ethylmorphine, mefenamic acid, methamphetamine, morphine, phenylbutazone, salicylic acid

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**KEY WORDS**

also details of isocratic elution

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**REFERENCE**Hill,D.W. Evaluation of alkyl bonded silica and solvent phase modifiers for the efficient elution of basic drugs on HPLC, *J.Liq.Chromatogr.*, **1990**, 13, 3147–3175.

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**SAMPLE****Matrix:** solutions

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**HPLC VARIABLES****Column:** 150 × 4.6 5 µm Adsorbosphere C18 (PEEK column) (retention times are longer and peaks broader with stainless steel column)**Mobile phase:** MeCN:20 mM pH 3.2 KH<sub>2</sub>PO<sub>4</sub> 23.4:76.6 containing 0.05% nonylamine**Flow rate:** 1.2**Detector:** UV 214

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**CHROMATOGRAM****Retention time:** 11

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**OTHER SUBSTANCES****Simultaneous:** amitriptyline, desmethyldoxepin, desipramine, doxepin, loxapine, maprotiline, nortriptyline, trazodone

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**REFERENCE***Supelco Catalog*, **1993**, p. 440.

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**SAMPLE****Matrix:** solutions

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**HPLC VARIABLES****Column:** 250 × 4.6 Econosil C8**Mobile phase:** MeCN:buffer 30:70 (Buffer was 20 mM KH<sub>2</sub>PO<sub>4</sub> and 14 mM triethylamine adjusted to pH 3.0 with phosphoric acid.)

**Injection volume:** 20

**Detector:** UV 210

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**CHROMATOGRAM**

**Retention time:** 8.3

**Limit of quantitation:** < 1000 ng/mL

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**OTHER SUBSTANCES**

**Simultaneous:** amitriptyline, amoxapine, carbamazepine, nortriptyline

**Also analyzed:** doxepin, desipramine, protriptyline, cyclobenzaprine, maprotiline

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**KEY WORDS**

UV spectra given

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**REFERENCE**

Ryan, T.W. Identification and quantification of tricyclic antidepressants by UV-photodiode array detection with multicomponent analysis, *J.Liq.Chromatogr.*, **1993**, 16, 1545–1560.

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**SAMPLE**

**Matrix:** solutions

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**HPLC VARIABLES**

**Guard column:** 30 × 2.1 Spheri-5 RP-8

**Column:** 220 × 2.1 Spheri-5 RP-8

**Mobile phase:** Gradient. A was 0.08% diethylamine and 0.09% phosphoric acid in water, pH 2.3.

B was MeCN:water 90:10 containing 0.08% diethylamine and 0.09% phosphoric acid. A:B 95:5 for 2 min, to 0:100 over 15 min (?), maintain at 0:100 for 5 min.

**Column temperature:** 50

**Flow rate:** 0.5

**Detector:** UV 200

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**CHROMATOGRAM**

**Retention time:** 14

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**OTHER SUBSTANCES**

**Simultaneous:** desmethyldoxepin, doxepin, desipramine, nortriptyline, amitriptyline

**Also analyzed:** amphetamine, chlordiazepoxide, chlorpromazine, desalkylflurazepam, diazepam, diethylpropion, ephedrine, fenfluramine, flurazepam, mesoridazine, methamphetamine, norchlordiazepoxide, nordiazepam, oxazepam, phentermine, phenylpropanolamine, prazepam, promazine, thioridazine, thiothixene, trifluoperazine

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**REFERENCE**

*Rainin Catalog*, C1-94, **1994**, p. 7.24.

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**SAMPLE**

**Matrix:** solutions

**Sample preparation:** Prepare a 1 mg/mL solution in MeOH, inject a 5 µL aliquot.

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**HPLC VARIABLES**

**Column:** 250 × 4.6 5 µm Lichrosphere cyanopropyl

**Mobile phase:** Carbon dioxide:MeOH:isopropylamine 90:10:0.05

**Column temperature:** 50

**Flow rate:** 3

**Injection volume:** 5

**Detector:** UV 220

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**CHROMATOGRAM**

**Retention time:** 3.19

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**OTHER SUBSTANCES**

**Simultaneous:** benactyzine, buclizine, hydroxyzine, perphenazine, thioridazine, amitriptyline, desipramine, nortriptyline, protriptyline

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**KEY WORDS**

SFC; pressure 200 bar

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**REFERENCE**

Berger, T.A.; Wilson, W.H. Separation of drugs by packed column supercritical fluid chromatography. 2. Anti-depressants, *J.Pharm.Sci.*, **1994**, 83, 287-290.

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**SAMPLE**

**Matrix:** solutions

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**HPLC VARIABLES**

**Column:** 250 × 4.6 Zorbax RX

**Mobile phase:** Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

**Column temperature:** 30

**Flow rate:** 2

**Detector:** UV 210

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**OTHER SUBSTANCES**

**Also analyzed:** acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepam, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlor-diazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenopropfen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiaicol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, indomethacin, isocarboxystyryl, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephénytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyl dopa, methyl dopamine, methylphenidate, methylprednisolone, methyltestosterone, methypylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, nor-epinephrine, nortriptyline, noscapine, nyldrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phenacyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyridylidone, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sulfadiazine, sulfadimethoxine, sulfathiazole, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfanilamide, sulfapyridine, sulfasoxazole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole,

thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranlycypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripeleennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

## REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

## SAMPLE

**Matrix:** solutions

## HPLC VARIABLES

**Column:** 250 × 4.6 5 µm Supelcosil LC-DP (A) or 250 × 4.5 µm LiChrospher 100 RP-8 (B)

**Mobile phase:** MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)

**Flow rate:** 0.6

**Injection volume:** 25

**Detector:** UV 229

## CHROMATOGRAM

**Retention time:** 14.70 (A), 6.78 (B)

## OTHER SUBSTANCES

**Also analyzed:** acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clobazepam, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyrindamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephentyoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyl dopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, trifluorpromazine, trimetoprim, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

## KEY WORDS

details of plasma extraction

## REFERENCE

Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

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**SAMPLE****Matrix:** solutions**Sample preparation:** Prepare a 1-10 µg/mL solution in water, inject an aliquot.

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**HPLC VARIABLES****Column:** 250 × 4.6 5 µm Hypersil SCX/C18**Mobile phase:** MeCN:25 mM pH 3 Na<sub>2</sub>HPO<sub>4</sub> 50:50**Injection volume:** 20**Detector:** UV 254

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**CHROMATOGRAM****Retention time:** k' 3.37

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**OTHER SUBSTANCES****Also analyzed:** amitriptyline, barbital, benzoic acid, butabarbital, clomipramine, clonazepam, desipramine, diazepam, flurazepam, furosemide, nitrazepam, phenobarbital, phenol, phenolphthalein, pindolol, propranolol, resorcinol, salicylic acid, secobarbital, terbutaline, xylazine

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**KEY WORDS**

effect of mobile phase pH on capacity factor is discussed

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**REFERENCE**Walshe, M.; Kelly, M.T.; Smyth, M.R.; Ritchie, H. Retention studies on mixed-mode columns in high-performance liquid chromatography, *J. Chromatogr. A*, **1995**, 708, 31–40.

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**SAMPLE****Matrix:** solutions**Sample preparation:** Inject a 20 µL aliquot of a 100-500 µg/mL solution in mobile phase.

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**HPLC VARIABLES****Column:** 100 × 4.6 5 µm Hypersil C8 MOS 100A coated with phosphatidylcholine (95% pure soybean lecithin, Epikuron, Lucas Meyer & Co.) (Coat column by recycling a 1 mM solution of phosphatidylcholine in MeOH:water 80:20 for 24 h.)**Mobile phase:** MeCN:35 mM pH 7.4 sodium phosphate buffer 40:60**Flow rate:** 0.5–2**Injection volume:** 20**Detector:** UV 254

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**CHROMATOGRAM****Retention time:** k' 6.31

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**OTHER SUBSTANCES****Also analyzed:** amoxicillin, antipyrine, carbamazepine, chlorpheniramine, chlorpromazine, clonidine, codeine, desipramine, diphenhydramine, dipyrindamole, ephedrine, flufenamic acid, haloperidol, hydroxyzine, indomethacin, lidocaine, megestrol acetate, metoprolol, nabumetone, nadolol, phenobarbital, phenol, promazine, propranolol, pyrilamine, quinidine, ropinirole, testosterone, thioridazine, tolfenamic acid, verapamil**Noninterfering:** acetaminophen, aspirin, azathioprine, caffeine, carprofen, chlorambucil, cimetidine, fenoterol, flurbiprofen, ibuprofen, ketoprofen, ranitidine, salicylic acid, sulfamethoxazole, theophylline, thioguanine, tiaprofenic acid, trimethoprim, valproic acid

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**KEY WORDS**

comparison with capillary electrophoresis

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**REFERENCE**Hanna, M.; de Biasi, V.; Bond, B.; Salter, C.; Hutt, A.J.; Camilleri, P. Estimation of the partitioning characteristics of drugs: A comparison of a large and diverse drug series utilizing chromatographic and electrophoretic methodology, *Anal. Chem.*, **1998**, 70, 2092–2099.

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**SAMPLE****Matrix:** urine

**Sample preparation:** 500  $\mu$ L Urine + N-ethylnordiazepam + chlorpheniramine + 100  $\mu$ L buffer, centrifuge at 11000 g for 30 s, inject a 500  $\mu$ L aliquot onto column A with mobile phase A, after 0.6 min backflush column A with mobile phase A to waste for 1.6 min, elute column A with 250  $\mu$ L mobile phase B, with 200  $\mu$ L mobile phase C, and with 1.15 mL mobile phase D. Elute column A to waste until drugs start to emerge then elute onto column B. Elute column B to waste until drugs started to emerge, then elute onto column C. When all the drugs have emerged from column B remove it from the circuit, elute column C with mobile phase D, monitor the effluent from column C. Flush column A with 7 mL mobile phase E, with mobile phase D, and mobile phase A. Flush column B with 5 mL mobile phase E then with mobile phase D. (Buffer was 6 M ammonium acetate adjusted to pH 8.0 with 2 M KOH.)

#### HPLC VARIABLES

**Column:** A  $10 \times 2.1$  12-20  $\mu$ m PRP-1 spherical poly(styrene-divinylbenzene) (Hamilton); B  $10 \times 3.2$  11  $\mu$ m Aminex A-28 (Bio-Rad); C  $25 \times 3.2$  5  $\mu$ m C8 (Phenomenex) +  $150 \times 4.6$  5  $\mu$ m silica (Macherey-Nagel)

**Mobile phase:** A 0.1% pH 8.0 potassium borate buffer; B 6 mM  $\text{KH}_2\text{PO}_4$  containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid; C MeCN:buffer 40:60 (Buffer was 6 mM  $\text{KH}_2\text{PO}_4$  containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.); D MeCN:buffer 33:67 (Buffer was 6 mM  $\text{KH}_2\text{PO}_4$  containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.); E MeCN:buffer 70:30 (Buffer was 6 mM  $\text{KH}_2\text{PO}_4$  containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.)

**Column temperature:** ambient (column A), 40 (columns B and C)

**Flow rate:** A 5; B-E 1

**Injection volume:** 500

**Detector:** UV 210, UV 235

#### CHROMATOGRAM

**Retention time:** k' 4.2

**Internal standard:** N-ethylnordiazepam (k' 2.1), chlorpheniramine (k' 5.9)

**Limit of detection:** 300 ng/mL

#### OTHER SUBSTANCES

**Extracted:** morphine, codeine, hydromorphone, hydrocodone, caffeine, cotinine, benzoylecgonine, secobarbital, oxazepam, phenobarbital, nordiazepam, diazepam, phenylpropanolamine, phentermine, amphetamine, phenmetrazine, lidocaine, ephedrine, pentazocine, methamphetamine, desipramine, nortriptyline, diphenhydramine, methadone

**Interfering:** flurazepam, amitriptyline

#### KEY WORDS

column-switching

#### REFERENCE

Binder, S.R.; Regalia, M.; Biaggi-McEachern, M.; Mazhar, M. Automated liquid chromatographic analysis of drugs in urine by on-line sample cleanup and isocratic multi-column separation, *J. Chromatogr.*, **1989**, 473, 325-341.

#### SAMPLE

**Matrix:** vitreous humor

**Sample preparation:** 600  $\mu$ L Vitreous humor + 3 mL 0.1 M NaCl + 50  $\mu$ L 4  $\mu$ g/mL desmethyl-clomipramine in water, mix for a few s, add to a C18 SepPak attached to a 5 mL syringe, allow to flow through (10-15 min). Wash with 1 mL 0.1 M NaCl, wash with 1 mL water, wash 3 mL reagent by gravity. Elute with 3 mL MeOH and push air through to remove as much as possible. Evaporate in vacuum at 37°, vortex with 50  $\mu$ L mobile phase for 1 min, inject 25  $\mu$ L aliquot. (Reagent was isopropanol:n-heptane:1 M sulfuric acid 40:320:1.)

#### HPLC VARIABLES

**Guard column:**  $50 \times 4.6$  30  $\mu$ m Permaphase ETH

**Column:**  $250 \times 4.6$  5-6  $\mu$ m Zorbax cyanopropyl

**Mobile phase:** MeCN:0.5 M acetic acid:n-butylamine 40:60:0.0022

**Flow rate:** 2.5

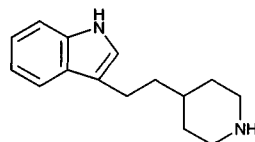
**Injection volume:** 25

**Detector:** UV 254

**CHROMATOGRAM****Retention time:** 22**Internal standard:** Desmethyldomipramine**Limit of detection:** 16.7 ng/mL**OTHER SUBSTANCES****Simultaneous:** amitriptyline, doxepin, nortriptyline, metabolites**Noninterfering:** acetaminophen, N-acetylprocainamide, amikacin, caffeine, carbamazepine, chloramphenicol, clonazepam, cyclosporine, diazepam, digoxin, disopyramide, ethosuximide, flurazepam, gentamicin, haloperidol, kanamycin, lidocaine, meprobamate, methapyriline, methaqualone, methotrexate, methpyrion, netilmicin, pentazocine, pentobarbital, phenobarbital, phenytoin, prazepam, primidone, procainamide, propranolol, quinidine, salicylic acid, secobarbital, streptomycin, theophylline, tobramycin, tocainide, valproic acid, vancomycin.**REFERENCE**

Evanson, M.A.; Engstrand, D.A. A SepPak HPLC method for tricyclic antidepressant drugs in human vitreous humor, *J. Anal. Toxicol.*, **1989**, *13*, 322–325.

# Indalpine

**Molecular formula:** C<sub>15</sub>H<sub>20</sub>N<sub>2</sub>**Molecular weight:** 228.34**CAS Registry No.:** 63758-79-2**Merck Index:** 4965**SAMPLE****Matrix:** blood**Sample preparation:** 2 mL Plasma + 100 µL 1 µg/mL IS in MeOH + 200 µL 5 M NaOH, vortex for 1 min, add 4 mL dichloromethane, shake for 15 min, centrifuge at 3000 g for 10 min, repeat extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 38°, reconstitute the residue in 50 µL mobile phase, vortex, centrifuge at 3000 g for 5 min, inject a 10 µL aliquot.**HPLC VARIABLES****Guard column:** 20 × 3 Bondapak C18/Corasil**Column:** 300 × 3.9 10 µm µBondapak C18**Mobile phase:** MeOH:10 mM K<sub>2</sub>HPO<sub>4</sub>:acetic acid 50:50:1**Flow rate:** 1**Injection volume:** 10**Detector:** F ex 220 em 370**CHROMATOGRAM****Retention time:** 8**Internal standard:** 4-[(5-methoxy-3-indolyl)methyl]piperidine (PK 26042) (5)**Limit of detection:** 2 ng/mL**OTHER SUBSTANCES****Extracted:** metabolites**KEY WORDS**

plasma; pharmacokinetics

**REFERENCE**

Jozefczak, C.; Ktorza, N.; Uzan, A. High-performance liquid chromatographic determination of indalpine, a new non-tricyclic antidepressant, in human plasma: identification and simultaneous measurement of its major plasma metabolite, *J. Chromatogr.*, **1982**, *230*, 87–95.



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**SAMPLE****Matrix:** blood, tissue**Sample preparation:** 200  $\mu$ L Plasma + 10  $\mu$ L 1  $\mu$ g/mL IS in MeOH + 50  $\mu$ L 1 M NaOH + 4 mL dichloromethane, shake on an alternating agitator for 20 min, centrifuge at  $-4^{\circ}$  at 2000 g for 15-20 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at  $38^{\circ}$ , reconstitute the residue in 100  $\mu$ L mobile phase, inject a 50  $\mu$ L aliquot. Tissue. Mouse brain + 25  $\mu$ L 1  $\mu$ g/mL IS in MeOH + 3 mL 1 M perchloric acid, homogenize in a potter apparatus (Ultraturax), centrifuge at 2000 g for 15 min. Remove the supernatant and adjust the pH to 12 with 1 M NaOH, add 4 mL dichloromethane, shake on an alternating agitator for 20 min, centrifuge at  $-4^{\circ}$  at 2000 g for 15-20 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at  $38^{\circ}$ , reconstitute the residue in 100  $\mu$ L mobile phase, inject a 50  $\mu$ L aliquot.

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**HPLC VARIABLES****Column:** 150  $\times$  3.9 5  $\mu$ m Nova-Pack C18**Mobile phase:** MeOH:water:5 mM hexanesulfonic acid 52:48:2**Flow rate:** 0.8**Injection volume:** 50**Detector:** E, Metrohm 641 VA, 1000 mV, Ag/AgCl reference electrode

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**CHROMATOGRAM****Retention time:** 5.8**Internal standard:** 5-methoxy-3-(4-piperidylmethyl)indole (3.6) (structure shown is 3-(4-piperidylmethyl)indole)**Limit of detection:** 2 ng/mL

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**OTHER SUBSTANCES****Extracted:** metabolites

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**KEY WORDS**

mouse; plasma; brain; pharmacokinetics

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**REFERENCE**Joulin,Y.; Doare,L.; Diquet,B. Micromethod for the determination of indalpine in mouse plasma using high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.*, **1986**, 381, 457-463.

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**SAMPLE****Matrix:** solutions**Sample preparation:** Prepare a solution in MeOH, inject an aliquot.

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**HPLC VARIABLES****Column:** 125  $\times$  4.9 5  $\mu$ m Spherisorb C8**Mobile phase:** MeCN:MeOH:buffer 13:35:52 (Buffer was 6.5 g/L (?)  $\text{KH}_2\text{PO}_4$  adjusted to pH 3 with orthophosphoric acid.)**Column temperature:** 45**Flow rate:** 1.2**Injection volume:** 60**Detector:** UV 254

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**CHROMATOGRAM****Retention time:** 2.9

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**OTHER SUBSTANCES****Simultaneous:** amitriptyline, bromazepam, chlorpromazine, clobazam, clomipramine, desipramine, diazepam, flunitrazepam, haloperidol, imipramine, levomepromazine, lorazepam, maprotiline, metapramine, mianserin, oxazepam, prochlorperazine, triazolam**Noninterfering:** meprobamate

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**REFERENCE**Rouquette,C.; Hecquet,D.; Pommereau,X.; Gardere,J.J.; Brachet-Liermain,A. Metapramine overdose: report of two cases and analytical determinations, *J.Anal.Toxicol.*, **1985**, 9, 275-277.

# Indapamide

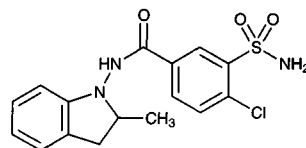
**Molecular formula:** C<sub>16</sub>H<sub>16</sub>ClN<sub>3</sub>O<sub>3</sub>S

**Molecular weight:** 365.84

**CAS Registry No.:** 26807-65-8

**Merck Index:** 4969

**Lednicer No.:** 2 349



## SAMPLE

**Matrix:** solutions

**Sample preparation:** Prepare a 10 µg/mL solution in MeOH, inject a 20 µL aliquot.

## HPLC VARIABLES

**Column:** 125 × 4.9 Spherisorb S5W silica

**Mobile phase:** MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7

**Flow rate:** 2

**Injection volume:** 20

**Detector:** E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

## CHROMATOGRAM

**Retention time:** 1.0

## OTHER SUBSTANCES

**Also analyzed:** acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzotamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazepine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyrindamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamlamine, meclophenoxate, meclizine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypropazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxymetazine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, pimindine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thiopropazine, thioridazine, thiothixene, thonzylamine, timolol, tocanide, tolpropamine, tolycaine, tranlycypromine, trazodone, trifluoperazine, trifluoperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripeleminamine, triprolidine, tryptamine, verapamil, xylometazoline

**REFERENCE**

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, 323, 191-225.

# Indeloxazine hydrochloride

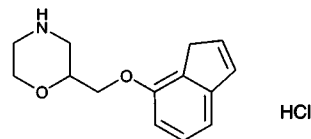
**Molecular formula:**  $C_{14}H_{18}ClNO_2$

**Molecular weight:** 267.76

**CAS Registry No.:** 65043-22-3

**Merck Index:** 4972

**Lednicer No.:** 4 59

**SAMPLE**

**Matrix:** blood

**Sample preparation:** 1 mL Plasma + 1 mL 300 ng/mL viloxazine in water + 500  $\mu$ L 200 mM ammonium hydroxide + 4 mL ether, extract, centrifuge. Remove the organic layer and evaporate it to dryness at 45°, reconstitute the residue in 100  $\mu$ L 3 mg/mL sodium bicarbonate and 200  $\mu$ L 125  $\mu$ g/mL dansyl chloride in acetone, heat at 45° for 20 min, cool, add 4 mL ether. Wash the ether solution twice with 3 mL water and evaporate it to dryness at 45°. Take up the residue in 100  $\mu$ L n-heptane, inject a 3-5  $\mu$ L aliquot.

**HPLC VARIABLES**

**Column:** 150  $\times$  4.5  $\mu$ m LiChrosorb SI-60

**Mobile phase:** n-Heptane:ethyl acetate 20:3

**Flow rate:** 1.5

**Injection volume:** 3-5

**Detector:** F ex 365 em 505

**CHROMATOGRAM**

**Retention time:** 3

**Internal standard:** viloxazine (4)

**Limit of detection:** 5 ng/mL

**KEY WORDS**

plasma; normal phase; derivatization

**REFERENCE**

Kamimura,H.; Sasaki,H.; Yokoi,K.; Kawamura,S. Determination of indeloxazine in plasma by liquid chromatography and gas chromatography-mass spectrometry, *J.Pharm.Sci.*, **1985**, 74, 559-561.

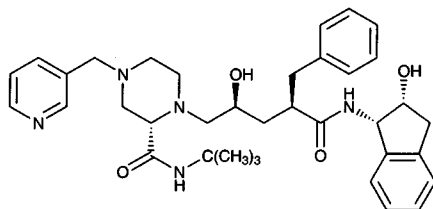
# Indinavir

**Molecular formula:**  $C_{36}H_{47}N_5O_4$

**Molecular weight:** 613.80

**CAS Registry No.:** 150378-17-9, 157810-81-6 (sulfate)

**Merck Index:** 4979

**SAMPLE**

**Matrix:** blood

**Sample preparation:** 300  $\mu$ L Plasma + 300  $\mu$ L 50 mM pH 9.0 ammonium dihydrogen phosphate buffer + 30  $\mu$ L 10.5  $\mu$ g/mL IS in water, vortex for 10 s, add 3 mL diethyl ether, vortex for 30